

Abstract

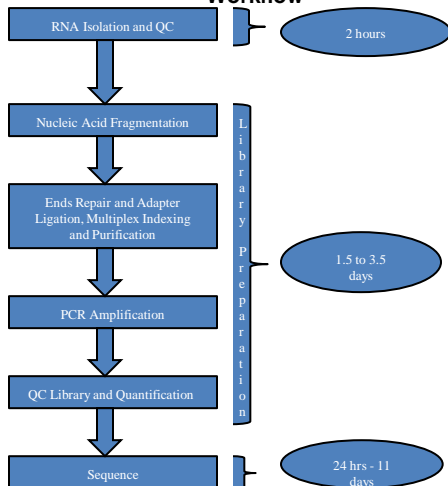
The Uganda Virus Research Institute (UVRI) is a national reference laboratory for viral infections including HIV, Influenza and Hemorrhagic fevers. Next generation sequencing (NGS) is being incorporated into laboratory diagnosis for enhanced pathogen detection during epidemic outbreak investigations. To this end, a regional genome center with capacity for deep sequencing is being established at the UVRI National Influenza Center (NIC) with assistance from various collaborating and development partners including the Los Alamos National laboratory, The Defence Threat Reduction Agency (DTRA), and Centers for Disease Control and Prevention (CDC). An Illumina MiSeq sequencer with complementary high-throughput computing infrastructure to support bioinformatic analysis has been installed at the institute. In addition, specialized training of 3 core staff in sample processing, sequencing and data analysis is being provided.

We hereby present site infrastructural developments, NGS protocols and data from a recent meta-transcriptomic analysis of four outbreak samples run at the facility.

Methods

Four whole blood samples from patients presenting with various viral hemorrhagic fever (VHF) symptoms, and previously tested for Ebola virus (EBV) Rift Valley Fever Virus (RVF), Crimean Congo Hemorrhagic Fever Virus (CCHFV), Yellow Fever Virus (YFV), West Nile Virus (WNV), Chikungunya Virus (CHIKV), Dengue Virus (DENV), Zika Virus (ZIKAV) by Elisa and PCR, were processed for transcriptome sequencing.

Fig 1. Schematic Illumina Sequencing Workflow



Methods cont....

Bioinformatics analysis:

FASTQ Miseq output files were imported into CLC workbench Ver 7.5.1 for:

- Data QC,
- Removal of host and adapter sequences
- Removal of poor quality sequences (>0.01 Q-score limit) and short reads (<50bases))
- Community profiling

Fig 2. The UVRI genomic center Bioinformatics computer cluster



Results

ARBO_0468UVRI

NCBI-Reference	Genes mapping to reference	% of total clean reads [9,307,696]	Organism Name	Medical Importance
NC_018220	1098	0.01	<i>Pseudomonas putida</i>	Usually non pathogenic
NC_010831	605	0.01	<i>Chlorobium phaeobacteroides</i>	"
NC_008543	430	<0.01	<i>Burkholderia cenocepacia</i>	"
NC_022098	320	"	<i>Pandoravirus salinus</i>	"
NC_001493	225	"	<i>Ictalurid herpesvirus 1</i>	Catfish virus
NC_006351	183	"	<i>Burkholderia pseudomallei</i>	melioidosis
NC_002967	179	"	<i>Treponema denticola</i>	Associated with periodontal disease
NC_008912	173	"	<i>Glypta fulmerianae ichnovirus</i>	"
NC_007346	167	"	<i>Emiliania huxleyi virus</i>	algal virus
NC_013715	162	"	<i>Rothia mucilaginosa</i>	"
NC_013520	106	"	<i>Veillonella parvula</i>	" (Part of oral flora)
NC_013853	100	"	<i>Streptococcus mitis</i>	Usually non pathogenic

ARBO_0473UVRI

NCBI-Reference	Genes mapping to reference	% of total clean reads [15,029,010]	Organism Name	Medical Importance
NC_018220	1716	0.01	<i>Pseudomonas putida</i>	Non pathogenic
NC_013715	932	<0.01	<i>Rothia mucilaginosa</i>	"
NC_022098	664	"	<i>Pandoravirus salinus</i>	"
NC_010831	467	"	<i>Chlorobium phaeobacteroides</i>	"
NC_008543	394	"	<i>Burkholderia cenocepacia</i>	"
NC_013853	384	"	<i>Streptococcus mitis</i>	"
NC_015964	353	"	<i>Haemophilus parainfluenzae T3T1</i>	Pneumonia
NC_008912	314	"	<i>Glypta fulmerianae ichnovirus</i>	non pathogenic
NC_013520	300	"	<i>Veillonella parvula</i>	part of oral flora
NC_020515	287	"	<i>Bifidobacterium bifidus</i>	Sheep pathogen
NC_014643	247	"	<i>Rothia dentocariosa</i>	normal microbes in mouth
NC_001493	240	"	<i>Ictalurid herpesvirus 1</i>	Catfish pathogen
NC_015291	219	"	<i>Streptococcus oralis</i>	Commensal of oral cavity
NC_002967	172	"	<i>Treponema denticola</i>	see earlier
NC_007346	140	"	<i>Emiliania huxleyi virus</i>	see earlier
NC_006351	128	"	<i>Burkholderia pseudomallei</i>	see earlier
NC_012913	112	"	<i>Agrobacterium tumefaciens</i>	soil flora
NC_020833	100	"	<i>Mycoplasma haemolytica</i>	Cattle respiratory pathogen

Results cont...

ARBO_0474UVRI

NCBI-Reference	Genes mapping to reference	% of total clean reads [8,601,186]	Organism Name	Medical Importance
NC_018220	636	0.01	<i>Pseudomonas putida</i>	See earlier
NC_010831	260	<0.01	<i>Chlorobium phaeobacteroides</i>	"
NC_008543	227	"	<i>Burkholderia cenocepacia</i>	"
NC_022098	146	"	<i>Pandoravirus salinus</i>	"
NC_001493	108	"	<i>Ictalurid herpesvirus 1</i>	Catfish virus
NC_002967	103	"	<i>Treponema denticola</i>	See earlier
NC_007346	92	"	<i>Emiliania huxleyi virus</i>	"
NC_006351	70	"	<i>Burkholderia pseudomallei</i>	Earlier described
NC_008912	67	"	<i>Glypta fulmerianae ichnovirus</i>	"

ARBO_0478UVRI

NCBI-Reference	Genes mapping to reference	% of total clean reads [1,103,988]	Organism Name	Medical Importance
NC_013715	1098	0.1	<i>Rothia mucilaginosa</i>	See earlier
NC_013520	605	0.05	<i>Veillonella parvula</i>	see earlier
NC_013853	430	0.04	<i>Streptococcus mitis</i>	see earlier
NC_008543	320	0.03	<i>Burkholderia cenocepacia</i>	see earlier
NC_014643	225	0.02	<i>Rothia dentocariosa</i>	see earlier
NC_015964	183	0.02	<i>Haemophilus parainfluenzae T3T1</i>	Pneumonia
NC_015291	179	0.02	<i>Streptococcus oralis</i>	oral commensal
NC_002967	173	0.02	<i>Treponema denticola</i>	see earlier
NC_006351	167	0.02	<i>Burkholderia pseudomallei</i>	see earlier
NC_012913	162	0.01	<i>Agrobacterium tumefaciens</i>	see earlier
NC_012803	106	<0.01	<i>Micrococcus luteus</i>	Usually non pathogenic
NC_014370	100	"	<i>Prevotella melanogena</i>	Opportunistic

Discussion/Conclusion

UVRI Genome Center

Facility is functional though some operational challenges still remain

Outbreak diagnosis

▪ Metagenomic analysis from the 4 samples showed no substantial gene expression from known agents causing VHF or severe clinical disease, other than ARBO_0478 for H. parainfluenzae

▪ Supplementary diagnosis from other sample types/and or more in-depth bioinformatics data manipulations could improve accuracy of this analysis

Acknowledgments

